



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Application No. : 09/036,724  
Applicant : Soldano Ferrone et al.  
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Commissioner for Patents  
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**DECLARATION UNDER 37 C.F.R. § 1.132 OF**  
**DANIEL J. HICKLIN, PH.D.**

I, Daniel J. Hicklin, Ph.D. hereby declare that:

1. I am a named co-inventor on the above-identified patent application.
2. Using guidelines set forth in the specification of the above-identified application, I and co-inventor Soldano Ferrone, or those working under our direction and supervision, performed the following experiments and obtained the results described below.

**Dendritic Cells (DC)**

DC were isolated using the following protocol. Bone marrow from C57BL6 mice was depleted of red blood cells by treatment in 0.5% ammonium chloride, followed by treatment with a combination of monoclonal antibodies specific for CD4, CD8, Ia,

granulocytes and then with rabbit complement. The remaining cells were cultured in RPMI 1640 containing 10% FBS as well as GM-CSF and IL-4. Three days later, nonadherent cells were discarded and adherent cells cultured for 4 more days. Adherent cells were then transferred to a new plate and cultured for another 3 days before harvesting for use as antigen presenting cells.

#### **Pulsing of DC with Flk-1AP Antigen**

DC were washed twice in serum-free AIMV media and incubated overnight in AIMV with 100 µg/ml of an affinity-purified fusion protein of Flk-1 and alkaline phosphatase (Flk-1AP). The cells were then washed twice in AIMV before being used for vaccination.

#### **Vaccination of Mice with DC Pulsed with Flk-1AP**

Lewis lung carcinoma metastasis model was used to assess the antitumor effect of vaccination with DC pulsed with Flk-1AP. Briefly, C57BL6 mice were injected either intravenously or intraperitoneally, with each mouse receiving  $1 \times 10^5$  Flk-1 AP-pulsed DC at Day 0. Seven days later, each mouse was inoculated intra-footpad with  $1 \times 10^5$  D122 cells. Visible tumors were surgically removed at day 10. During this period, mice received two boost vaccinations every ten days. At Day 60, mice were sacrificed and lungs removed for weighing and assessment of metastases. As a control, a group of mice were vaccinated with DC alone.

#### **Inhibition of Tumor Metastases**

Mice vaccinated with DC pulsed with Flk-1AP developed a significantly smaller number of tumors in the lungs compared to the groups vaccinated with DC alone. Comparably, the average weight of the lungs from the Flk-AP group was significantly lower than the controls. In both instances, the number of tumors present and the weight of

the lungs of mice vaccinated with DC pulsed with Flk-1AP was less than half of the control group.

3. I declare that all of the above statements made based on my own knowledge are true and that all of the above statements that were made on information and belief are believed to be true, and further declare that these statements are made with the knowledge that knowing, willful and materially false statements, as are defined under Section 1001 of Title 18 of the United States Code, are punishable by fine, imprisonment, or both under Section 1001, and that such statements may affect the validity of a United States Patent that issues from the above captioned patent application.

Date: 11/29/07



Daniel J. Hicklin, Ph.D.